Systematics and the analysis of integumental lipids: the uropygial gland

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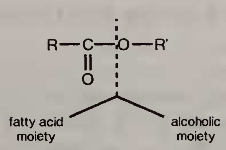
Whilst classification of birds has been exclusively deduced from anatomical, morphological and ethological criteria up to the middle of this century, more recently it became obvious that chemical data also may be used to study the relationships between species. Since DNA codes for all genetically dependent properties, its precise analysis could give us the best answer to questions on classification. Attempts have been made accordingly to use DNA analysis for this purpose, although the method suffers from the fact that no definite structures can presently be obtained, since only the extent of more or less identical sequences may be calculated from the data generated. Nevertheless, Sibley and co-workers have suggested an avian system based upon the so-called DNA/DNA-hybridization experiments (Sibley & Ahlquist 1990).

Since DNA, of course, generally codes for the entire chemistry coursing in the organism which depends on the enzymatic equipment, the analysis of the enzymatic activities being responsible for these processes could also be used for chemotaxonomic attempts (protein patterning). Before DNA hybridization techniques became available, protein patterning had been used in this way by various authors (e.g. Sibley 1970, Sibley & Ahlquist 1972).

Proteins are the final determinant of the formation of the various endogenous as well as the externally released secretions. These secondary metabolites also reflect the enzyme equipment of organisms to a certain extent, although chemotaxonomists using this method must be aware that they are looking through a necessarily small window into the complex reality of life.

The pattern of the lipid constituents formed in the uropygial gland (preen gland) of birds has been found to be very characteristic for a species and fortunately differs markedly between various taxa (Jacob 1978, Jacob & Ziswiler 1982). This has led to a broader investigation of these lipids which may contribute to our understanding of relationships among avian species. Some of these results along with some hitherto unpublished data are reviewed here.

It has been found that preen gland secretions, being the main sources of avian integumental lipids in most instances, consist of monoester waxes, which are composed of a fatty acid and a monohydric alcoholic moiety. The structure may be condensed to the formula:



with R and R′ standing for an alkyl chain $-C_nH_{2n+1}$.

Only few exceptions to this finding have been reported. For instance, in galliform and apterygiform species diols have been found instead of monohydric alcohols. Occasionally also, fatty acids may be replaced by hydroxy acids, as found e.g. in the case of pigeons and in some Ciconiiform species (*Ciconia ciconia*—Jacob 1976, *C. nigra*—Jacob, unpubl.).

Uropygial lipids in systematics

Meanwhile some 500 different species have been analyzed for the chemical composition of their uropygial lipids and a broad structural variety of the above waxes has been detected. Both of the wax moieties may vary in 4 respects:

(1) *variation of the chain length*; i.e. 'n' in the above $-C_nH_{2n+1}$ may stand for odd and even numbers from about 6 up to 30;
(2) *variation of the location of the substituent*; i.e. substituents may be located at various carbon atoms. Biochemically there are more basic differences between 2 waxes, one being branched at even and the other at odd carbon atoms compared to 2 waxes both branched at 2 different but even carbon atoms;
(3) *variation of the degree of substitution*; i.e. R and R′ may be more or less alkyl-branched resulting in mono, di-, tri-, tetra- or even penta-methyl-branched acidic and/or alcoholic moieties (e.g. in *Alcedo atthis*);
(4) *variation of the substituent*; i.e. the substituent may be a methyl- ($-CH_3$), an ethyl- ($-C_2H_5$), a propyl- ($-C_3H_7$) or a butyl-group ($-C_4H_9$).

As an example, monoester waxes composed of 3-methyl-substituted fatty acids and 3-methyalkanols have been found as main constituents of the preen wax from finches (Fringillidae), whereas 2-ethyl-substituted fatty acids and unbranched alcohols are structural elements in the case of wrens (Troglodytidae) (Fig. 1.)

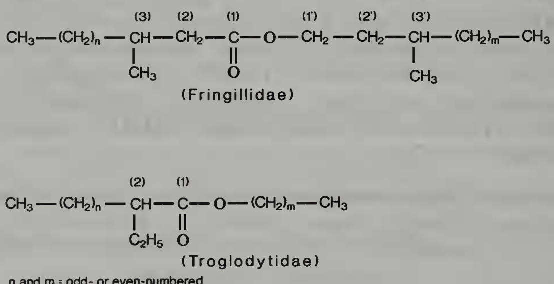


Figure 1. Examples of different structures of monoester waxes found in finches and in wrens.

Because of the obviously unlimited combination of these structural moieties, an immense number of different final structures may be expected, most if not all of which are actually realized in nature. From this it would not be surprising if each bird had its own specific wax pattern.

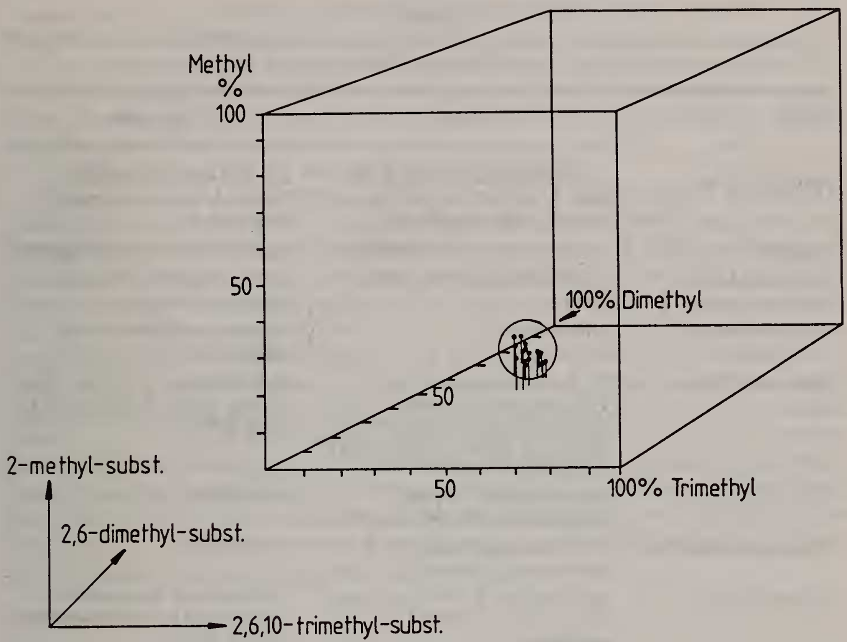


Figure 2. Intraspecific variation of the quantitative composition of wax acids from the uropygial gland secretion of the Blackbird *Turdus merula* (n = 18).

As we know now, however, this is definitely not the case. Prior to chemotaxonomic considerations, the intraspecific variation of the lipid composition has to be checked. This has been done for a number of species and the variation is found to be very limited, as may be seen from Fig. 2, with *Turdus merula* as as example. The circle surrounding the cloud of single points in this figure may be taken as a measure of the degree of intraspecific variation and it is the size of this cloud which decides whether 2 species can be separated by this method. Individuals of a species and its subspecies or races cannot be distinguished from each other by this method. Within different genera, only quantitative differences are found, which in many cases, however, are not significant and hence often cannot be used for classification. *Qualitative* structural differences may be expected at the family level, where at least quantitatively different patterns are observed. Orders may be distinguished by their qualitatively different patterns.

In Table 1 the specific characters for a number of orders are summarized. These data not only allow distinction between orders, but also seem to indicate relationships between some of them. For instance, it appears reasonable to assume that kiwis (Struthioniformes), tinamous (Tinamiformes) and galliform birds (Galliformes) form a group of related orders on the basis of the occurrence of alkane-2,3-diols in all of them. Similarly, Phoenicopteriformes and Anseriformes have many typical

TABLE 1
Typical structural characters found in the uropygial gland waxes in species from different Orders of birds

Order	Acid	Alcohols
Sphenisciformes	complex mixture of 2-; 3-; 4-; 2,x-; 3,x-; 4,x-; 2,x,y-; 3,x,y-methyl-substituted acids	2-; 3-; 4-; 2,x-; 3,x-; 4,x-; 2,x,y-; 3,x,y-; 4,x,y,-methyl-substituted
Procellariiformes	similar to the Sphenisciformes	similar to the Sphenisciformes
Struthioniformes (Apterygiformes)	unbranched and 3-hydroxy acids	unbranched alkanols and alkane-2,3-diols
Tinamiformes	unbranched	unbranched alkanols and alkane-2,3-diols
Podicipediformes	3-; 2,x-; 3,x-; 2,x,y-; 3,x,y-; 2,x,y,z-; 3,x,y,z-methyl-substituted as well as 2-ethyl-; 2-ethyl-x-methyl- and 2-ethyl-x,y-dimethyl-substituted	unbranched, 2-; 4-; 2,x-; 4,x-; 2,x,y-; 4,x,y-; 2,x,y,z-methyl-substituted
Ciconiiformes	heterogenous, varying significantly between families	see acids
Phoenicopteriformes	2,6-; 4,6-; 2,x,y-methyl-substituted	unbranched
Anseriformes	unbranched, 2-; 4-; 2,x-; 4,x-; 2,x,y-; 2,x,y,z-methyl-substituted	unbranched; monomethyl-substituted at even-numbered carbon atoms
Falconiformes	2-; 2,x-; 2,x,y-methyl-substituted with the last substituent near the end of the molecule	unbranched; monomethyl-substituted at even-numbered carbon atoms
Gruiformes	2-; 4-; 2,x-; 4,x; 2,x,y; 4,x,y; 2,x,y,z- and 4,x,y,z-methyl-substituted with substituents located at every 4th carbon atom	mainly unsubstituted, otherwise monomethyl-substituted at even-numbered carbon atoms
Charadriiformes	2-; 4-; 2,x-; 4,x-; 2,x,y-methyl-substituted	mainly unsubstituted, otherwise monomethyl-substituted at even-numbered carbon atoms
Galliformes	unsubstituted	alkane-2,3-diols
Columbiformes	unbranched and 3-hydroxy acids	unsubstituted
Psittaciformes	unbranched, 2-, (w-1)- and (w-2)-methyl-substituted	mainly unsubstituted
Cuculiformes	mainly 3-methyl-substituted	mainly 3-methyl-substituted
Strigiformes	2-ethyl, 2-propyl- and 2-butyl-substituted	unbranched and monoethyl-substituted at even-numbered carbon atoms
Apodiformes	3-methyl-substituted	unbranched; 2- and 2,x-methyl-substituted
Piciformes	3-; 3,x-methyl-substituted	unbranched and 3-methyl-substituted
Passeriformes	heterogenous, varying significantly at the family level	see acids

constituents in common, and this holds also for the Sphenisciformes and the Procellariiformes.

Two examples may be given for the contribution of preen wax analysis to systematic questions: (1) the separation of the Tytonidae (barn owls) from the Strigiformes (owls) and (2) the systematic position of *Vultur gryphus* (the Condor).

Separation of the Tytonidae from the Strigidae

The chemical composition of the uropygial gland waxes of various owls (Jacob & Poltz 1974, Jacob & Hoerschelmann 1984) indicates that Strigiformes are characterized by the occurrence of 2-alkyl-substituted fatty acids with an ethyl-, propyl- and/or a butyl substituent, all very rare compounds, hardly any of which have been found anywhere else in nature. The alcoholic moiety is composed of unbranched and monomethyl-branched alkanols with a methyl substituent located at an even carbon atom. However, *Tyto* possesses an entirely different wax pattern composed of 3-methyl- and 3,x-dimethyl-substituted fatty acids and 3-, 3,x-, 4, and 4,x-methyl-substituted alcohols. Hence, Tytonidae may be readily distinguished from Strigidae by basic differences in their preen wax composition, as can be seen from Fig. 3. The barn owls (if they are owls at all) seem to link the owls with some other Orders such as Piciformes, Cuculiformes and Apodiformes, in which identical or at least very similar structures have been detected.

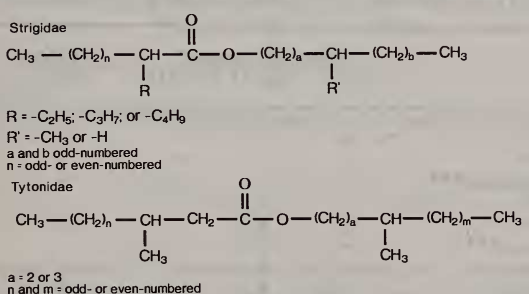


Figure 3. Chemical structures found in the preen waxes of Strigidae and Tytonidae.

The systematic position of the Condor Vultur gryphus

A nice example for an interdisciplinary approach to the solution of systematic problems has recently been given by comparing biochemical and ethological findings in the case of the Condor *Vultur gryphus* (Cathartidae). Although Beddard (1898) found similarities between Cathartidae and Ciconiiformes nearly 100 years ago, other biologists have generally associated *Vultur* with the birds of prey rather than with the Ciconiiform birds. König (1982) found support for Beddard's statement by studying the mating behaviour; and as presented in Figure 4, a comparative analysis of the preen wax composition of the Condor, storks and birds of prey clearly shows that identical wax patterns are synthesized in storks and in the Condor (Jacob 1983). These findings have now also been confirmed by DNA/DNA-hybridization experiments (Sibley & Ahlquist 1986).

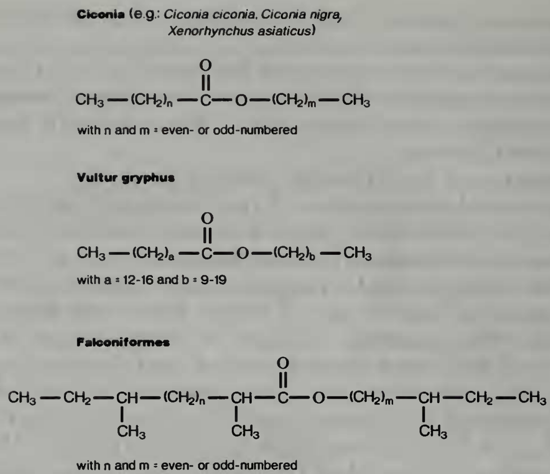


Figure 4. Chemical structures found in the preen waxes of *Vultur gryphus*, Ciconiiformes and Falconiformes.

TABLE 2

Preen wax types found in the uropygial gland of Ciconiiform birds

	Monoester waxes* type I	type II	type III	Diester waxes	Triglycerides
Ciconia ciconia	+	−	−	+	+
*Ciconia nigra****	+	−	−	+	+
*Xenorhynchus asiaticus****	+	−	−	−	−
*Egretta alba****	+	+	−	−	−
*Egretta sacra****	+	+	−	−	−
*Platalea leucorodia****	+	+	−	−	−
Scopus umbretta	+	+	−	−	−
Theristicus caudatus	(+)	+	−	−	−
Threskiornis aethiopicus	−	+	−	−	−
Ardea cinerea	+	−	−	−	+
*Ardea novaehollandiae****	+	+	+	−	−
Nycticorax nycticorax	+	−	+	−	−
*Botaurus poiciloptilus****	−	+	+	−	−
*Leptoptilos crumeniferus***	−	−	−	−	+

*type I: unbranched acids containing monoester waxes; type II; branched acids containing monoester waxes; type III: secondary alcohols containing monoester waxes
**feather lipids
***unpublished data

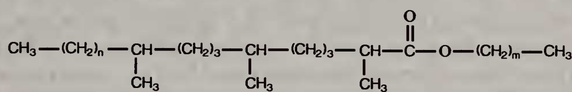
Ciconiiformes

In almost all Orders so far investigated, specific structures have been found so that species may be attributed to these taxa. There is, however, a remarkable exception to this in the case of the Ciconiiformes, where the composition of the waxes broadly varies. Not only the wax moieties but also the total lipid composition itself differs among the various families, in that monoester waxes are found in *Egretta, Threskiornis, Theristicus, Platalea* and *Scopus*, whereas additional diester waxes and triglycerides are detected in *Ciconia*. In Ciconiiformes also species exist which possess secondary alcohols as one wax moiety (*Botaurus, Nycticorax, Ardea*). The findings summarized in Table 2 may indicate a polyphyletic origin for the order Ciconiiformes.

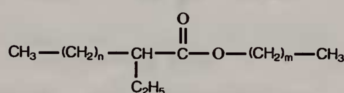
Passeriformes

From a chemotaxonomic viewpoint, the hitherto investigated species of the large order Passeriformes may be separated into at least 4 chemical groups according to their preen wax types:

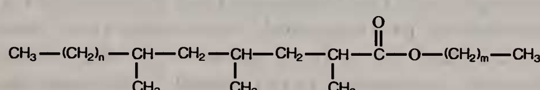
(1) waxes composed of unbranched alcohols and more or less highly methyl-branched acids substituted at every 4th carbon atom, with the first branch located at the 2- or 4-position.
The following families or subfamilies may be attributed to this group: the Tyrannidae, Funariidae of the Suboscines, and also the Corvidae, Alaudidae, Bombycillidae, Oriolidae, Monarchidae, Timaliinae, Pachycephalinae, Hirundinidae of the Oscines.



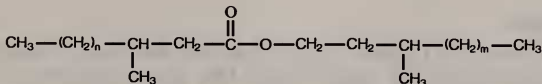
(2) waxes composed of unbranched alcohols and 2-ethyl-substituted fatty acids.
The Paridae and Troglodytidae may be combined in this group.



(3) waxes composed of unbranched alcohols and more or less highly branched fatty acids in which every 2nd carbon atom bears a methyl group, the first of which is located at carbon atom 2.
The Ploceidae and Estrildidae are found in this group.



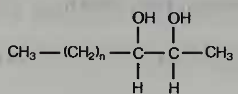
(4) waxes composed of 3-methylsubstituted alcohols and 3-methyl-substituted fatty acids.
To this group may be attributed the Fringillidae, Emberizidae, Passerinae, Motacillidae, Icteridae, Prunellidae, Carduelidae, Thraupidae.



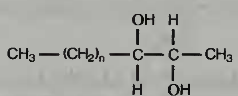
General considerations

Generally speaking, there are Orders which are characterized by a very broad spectrum of different lipid constituents and others in which highly specific structures are found. Very complex mixtures occur in Sphenisciformes, Procellariiformes and Podicipediformes. A smaller complex of different acids and alcohols are found in Gruiformes, Falconiformes, Strigiformes, Psittaciformes, Anseriformes and Phoenicopteriformes. In Galliformes, Coraciiformes, Cuculiformes, Piciformes and various Passeriformes the structural variation is even more restricted inasmuch as mostly only one homologous series of acids or alcohols participates in the spectrum of the preen wax. Extremely simple wax structures composed of only *one* acid and *one* alcohol have been detected in *Cinclus cinclus* (Bertelsen *et al.* 1975) and in many weaver-birds (e.g. in *Ploceus cucullatus, P. subaureus, P. galbula*—Poltz & Jacob 1973) indicating the presence of a highly specific enzyme system in these species.

Since weaver-birds are considered to be more recent steps in avian evolution, it appears that preen wax patterns become more specific and less complex with progressing speciation—a trend which has also been observed for the cuticular lipids of beetles (Jacob & Hanssen 1985). Under the assumption that this is a general principle, an interesting conclusion may be drawn for the order Galliformes. The preen waxes of species from this order are characterized by the occurrence of alkane-2,3-diols for which 2 stereoisomeric forms are known (*threo*- and *erythro*-)



erythro form



threo form

(with n = even- or odd-numbered

Mixtures of both forms have been found in *Gallus* and *Coturnix*, whereas *Leipoa, Meleagris* and *Perdix* possess only the *erythro*-forms. By contrast, *Phasianus colchicus* produces only one single diol, namely the *erythro*-octadecane-2,3-diol, i.e. a high stereo- and chain-length-specificity of the synthesizing enzyme(s) is expressed in this species. Following the hypothesis of a progressing chemical specialization, *Gallus* and *Coturnix* ought to be considered as ancient, *Leipoa, Meleagris* and *Perdix* as more recent forms and *Phasianus* as a preliminary endpoint of the evolution of the Galliformes. Within the closely related species *Gallus* and *Phasianus* and the couple *Coturnix* and *Perdix*, in both cases the second named are obviously the more modern forms.

Conclusions

Summarizing the results of preen gland analysis obtained from about 500 different species it seems that the wax composition is a helpful parameter for avian systematics. The slogan "Show me your preen gland secretion and I shall tell you who you are" certainly exaggerates and overestimates the significance of this character. Preen wax analysis, however, has contributed to the solution of several systematic questions and the data should be treated as one piece of a very complex puzzle.

References:

Beddard, F. E. 1898. *The Structure and Classification of Birds*. Longmans, Green, London.

Bertelsen, O., Eliasson, B., Odham, G. & Stenhagen, E. 1975. The chemical composition of the free-flowing secretion of the preen gland of the Dipper (*Cinclus cinclus*). *Chem. Scr.* 8: 5–7.

Jacob, J. 1976. Diester waxes containing 2-hydroxy fatty acids from the uropygial gland secretion of the White Stork (*Ciconia ciconia*). *Lipids* 11: 816–818.

— 1978. Uropygial gland secretions and feather waxes. Pp. 165–211 *in* A. H. Brush (ed.), *Chemical Zoology*, Vol. X.

— 1983. Zur systematischen Stellung von *Vultur gryphus* (Cathartiformes). *J. Orn.* 124: 83–86.

Jacob, J. & Hanssen, H.-P. 1985. Distribution and variability of cuticular hydrocarbons within the coleoptera. *Biochem. Syst. Ecol.* 14: 207–210.

Jacob, J. & Hoerschelmann, H. 1984. Chemotaxonomische Untersuchungen an Eulen (Strigiformes). *Funkt. Biol. Med.* 3: 56–61.

Jacob, J. & Poltz, J. 1974. Chemical composition of the uropygial gland secretions of owls. *J. Lipid Res.* 15: 243–248.

Jacob, J. & Ziswiler, V. 1982. The uropygial gland. Pp. 199–324 *in* D. S. Farner, J. R. King & K. C. Parkes (eds.), *Avian Biology*, Vol. VI. Academic Press, New York.

König, C. 1982. Zur systematischen Stellung der Neuweltgeier (Cathartidae). *J. Orn.* 123: 259–267.

Poltz, J. & Jacob, J. 1973. Bürzeldrüsensekrete von Webervögeln (Ploceidae). *Z. Naturforsch.* 28c: 449–452.

Sibley, C. G. 1970. A comparative study of the egg-white protein of passerine birds. Peabody Museum of Natural History. *Yale University, New Haven. Bull.* 32.

Sibley, C. G. & Ahlquist, J. E. 1972. A comparative study of the egg-white protein of non-passerine birds. Peabody Museum of Natural History. *Yale University, New Haven. Bull.* 39.

—, — 1986. Der DNA-Stammbaum der Vögel. *Spektr. Wissenschaft* (5/86): 96–107.

—, — 1990. *Phylogeny and Classification of Birds*. 976 pp. Yale University Press. New Haven & London.

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